Dkt. No.: 187894/US

## **AMENDMENTS TO THE CLAIMS**

The listing of claims will replace all prior versions, and listings, of claims in the application.

## **Listing of Claims:**

- 1. (Previously presented) Method for the preparation of a strain of evolved microorganisms for the production of 1,2-propanediol by the metabolism of a simple carbon source, said method comprising growing an initial bacterial strain, under selection pressure in an appropriate growth medium comprising a simple carbon source, said initial bacterial strain comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, in order to cause evolution, in said initial strain, of one or more genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes having an improved "1,2-propanediol synthase" activity, then selecting and isolating strain or strains of evolved\_micro-organisms having an improved "1,2-propanediol synthase" activity.
- 2. (Previously presented) The method of claim 1, wherein the gene involved in the conversion of methylglyoxal into lactate is selected from the group consisting in gloA, aldA and aldB.
- 3. (Previously presented) The method of claim 1, wherein the initial strain comprises deletion of the genes gloA, aldA, aldB and tpiA.
- 4. (Previously presented) The method of claim 1, wherein the initial strain comprises deletion of the genes *IdhA*, *pflA*, *pflB*, *adhE* and *edd*.

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5. (Previously presented) The method of claim 1, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.

- 6. (Previously presented) The method of claim 1, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
- 7. (Previously presented) The method of claim 5, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
- 8. (Previously presented) The method of claim 7, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
- 9. (Previously presented) The method of claim 6, wherein the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
- 10. (Previously presented) The method of claim 1, wherein one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone are introduced into the evolved microorganisms.
- 11. (Previously presented) The method of claim 10, wherein one the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate are from *C. acetobutylicum*.
- 12. (Previously presented) The method of claim 10, wherein the modified evolved strain comprising one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone is grown under selection pressure in an appropriate growth medium comprising a simple carbon source in order to cause, in said evolved modified evolved strain, the evolution of one or more genes involved in the conversion of acetyl-CoA and acetate to acetone towards an improved "acetone synthase" activity, the second generation of resulting evolved micro-organisms having an improved "1,2-propanediol synthase" activity and an improved "acetone synthase" activity are then selected and isolated.
- 13. (Previously presented) The method of claim 1, wherein the strain is selected from

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the group consisting of bacterium, a yeast and a fungus.

14. (Previously presented) The method of claim 13, wherein the strain is selected from the group consisting of *Escherichia* and *Corynebacterium*.

- 15. (Cancelled).
- 16. (Previously presented) Evolved strain that can be obtained by the method according to any of Claims 1.
- 17. (Original) Strain according to Claim 16, in which the gene *Ipd* has a point mutation whereby alanine 55 is replaced by valine.
- 18. (Previously presented) Method of preparation of 1,2-propanediol wherein an evolved strain of claim 16 is grown in an appropriate growth medium containing a simple carbon source, and wherein the 1,2-propanediol produced is recovered.
- 19. (Previously presented) The method of claim 18, wherein 1,2-propanediol and acetone are recovered.
- 20. (Previously presented) The method of claim 18, wherein 1,2-propanediol and/or acetone are purified.
- 21. (Previously presented) The method of claim 14, wherein the strain is selected among the group consisting of *E. coli*, and *C. glutamicum*.
- 22. (Previously presented) Initial bacterial strain of a microorganism comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate.
- 23. (Previously presented) The strain of claim 22, wherein the gene involved in the conversion of methylglyoxal into lactate is selected among the group consisting in *gloA*, *aldA* and *aldB*.
- 24. (Previously presented) The method of claim 22, wherein the initial strain comprises deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
- 25. (Previously presented) The strain of claim 22, wherein the initial strain comprises deletion of the genes *IdhA*, *pflA*, *pflB*, *adhE* and *edd*.
- 26. (Previously presented) The strain of claim 22, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
- 27. (Previously presented) The strain of claim 22, wherein the enzyme that favours

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NADH.

the metabolism of pyruvate into acetate has low sensitivity to inhibition by

- 28. (Previously presented) The strain of claim 27, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
- 29. (Previously presented) The strain of claim 27, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
- 30. (Previously presented) The strain of claim 22, selected from the group consisting of a bacterium, a yeast and a fungus.
- 31. (Previously presented) The strain of claim 30, selected from the group consisting of *Escherichia* and *Corynebacterium*.
- 32. (Previously presented) The strain of claim 16, comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, selected from the group consisting in *gloA*, *aldA* and *aldB*.
- 33. (Previously presented) The strain of claim 16, comprising deletion of the genes gloA, aldA, aldB and tpiA.
- 34. (Previously presented) The strain of claim 16, comprising deletion of the genes *IdhA*, *pflA*, *pflB*, *adhE* and *edd*.
- 35. (Previously presented) The strain of claim 16, comprising at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
- 36. (Previously presented) The strain of claim 36, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
- 37. (Previously presented) The strain of claim 36, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.(Previously presented) The strain of claim 37, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.

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(Previously presented) The strain of claim 36, wherein the enzyme that favours 38. the metabolism of pyruvate into acetate is an endogenous enzyme.

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- (Previously presented) The strain of claim 16, comprising one or more 39. heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone.
- (Previously presented) The strain of claim 40, wherein one the heterologous gene 40. or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate is from C. acetobutylicum.
- (Previously presented) The strain of claim 16, selected from the group consisting 41. of a bacterium, a yeast and a fungus.
- (Previously presented)] The strain of claim 16, selected from the group consisting 42. of Escherichia, and Corynebacterium.
- (Previously presented) The strain of claim 17, selected from the group consisting 43. of a bacterium, a yeast and a fungus.
- (Previously presented) The strain of claim 17, selected from the group consisting 44. of Escherichia, and Corynebacterium.
- (Previously presented) Evolved strain that can be obtained by the method of 45. Claim 10.
- (Previously presented) The strain of Claim 46, in which the gene Ipd has a point 46. mutation whereby alanine 55 is replaced by valine.
- (Previously presented) The strain of claim 46, selected from the group consisting 47. of a bacterium, a yeast and a fungus.
- (Previously presented) The strain of claim 46, selected from the group consisting 48. of Escherichia and Corynebacterium.